

Patent Claims

- 5 1. Variant of the major allergen Phl p 1 from timothy grass, characterised in that it has an additional Cys residue compared with the wild type.
2. Allergen variant according to Claim 1, characterised in that the additional Cys residue is located in the carboxyl-terminated region.
- 10 3. Allergen variant according to Claim 1 or 2, characterised in that the additional Cys residue is located in a higher position than amino acid position 140.
- 15 4. Allergen variant according to one or more of Claims Claim 1 to 3, characterised in that the additional Cys residue is located between amino acid positions 230 and 240.
- 20 5. Allergen variant according to one or more of Claims 1 to 4, characterised in that the additional Cys residue originates from an amino acid exchange.
- 25 6. Allergen variant rPhl p 1-A236C according to SEQ ID NO 2 according to one or more of Claims 1 to 5, characterised in that the additional Cys residue has been introduced by exchange of Ala 236.
- 30 7. DNA molecule which encodes for an allergen variant according to one or more of Claims 1 to 6.
8. DNA molecule according to SEQ ID NO 1 which encodes for the allergen variant according to Claim 6.

9. Process for the preparation of a variant of the recombinant major allergen rPhl p 1 according to one or more of Claims 1 to 6, characterised in that, by methods known per se,
- 5 - a base triplet encoding for a Cys residue is introduced the corresponding gene by insertion or exchange,
- the gene modified in this way is overexpressed in a host organism and
- 10 - the allergen variant obtained by overexpression is purified.
10. Process for the preparation and purification of a variant of the recombinant major allergen rPhl p 1 according to Claim 9 in soluble form, characterised in that the initially insoluble crude protein is denatured, subsequently renatured by dilution and purified by biochemical purification
- 15 steps.
11. Process for the purification of a variant of the recombinant major allergen rPhl p 1 according to Claim 9 in soluble form, characterised in that,
- 20 starting from the overexpressed, initially insoluble crude protein provided with an His tag for purification purposes, a plurality of biochemical purification steps, encompassing two-stage metal ion chelate affinity chromatography and the removal of the His tag, are carried out.
- 25
12. Allergen variant according to one or more of Claims 1 to 6, characterised in that it exists in various fold forms.
13. Fold form rPhl p 1-LM of the allergen variant according to one or more of Claims 1 to 6, obtainable by carrying out the following process steps:
- 30 - overexpression of the rPhl p 1 allergen variant provided with an His tag in a host organism,
- denaturing of the inclusion bodies isolated from the host organism
- 35 using guanidinium chloride
- renaturing of the dissolved protein on a chelate affinity chromatogra-

phy column

- removal of the His tag
- gel filtration
- 5 - further chelate affinity chromatography
- isolation of the target protein from the flow-through
- gel filtration.

10 14. Fold form rPhl p 1-HM of the allergen variant according to one or more of Claims 1 to 6, obtainable by carrying out the following process steps:

- overexpression of the rPhl p 1 allergen variant provided with an His tag in a host organism
- denaturing of the inclusion bodies isolated from the host organism
- 15 using guanidinium chloride
- renaturing of the dissolved protein on a chelate affinity chromatography column
- removal of the His tag
- 20 - gel filtration
- further chelate affinity chromatography
- elution of the target protein with an imidazole gradient
- gel filtration.

25 15. Allergen variant according to one or more of Claims 1 to 6 and 12 to 14 as medicament.

30 16. Use of an allergen variant according to Claim 15 and/or pharmaceutically usable derivatives thereof, including mixtures thereof in all ratios, for the preparation of a medicament for specific immunotherapy of allergies in the triggering of which the major allergen Phl p 1 from timothy grass is involved.

35 17. Pharmaceutical composition comprising an allergen variant according to Claim 15 and/or pharmaceutically usable derivatives thereof,

including mixtures thereof in all ratios, and, if desired, excipients and/or adjuvants.

5 18. Use of an allergen variant according to one or more of Claims 1 to 6 and 12 to 14 and/or derivatives thereof, including mixtures thereof in all ratios, for the *in vitro* diagnosis of allergies in the triggering of which the major allergen Phl p 1 from timothy grass is involved.

10 19. Recombinant DNA expression vector containing a DNA molecule according to Claim 7 or 8 for the treatment of allergies in the triggering of which the major allergen Phl p 1 from timothy grass is involved, by immunotherapeutic DNA vaccination.

15 20. Use of the expression vector according to Claim 19 and/or derivatives thereof, including mixtures thereof in all ratios, for the preparation of a medicament for the treatment of allergies in the triggering of which the major allergen Phl p 1 from timothy grass is involved, by immunotherapeutic DNA vaccination.

20 21. Pharmaceutical composition comprising an expression vector according to Claim 19 and/or pharmaceutically usable derivatives thereof, including mixtures thereof in all ratios, and, if desired, excipients and/or adjuvants, for the treatment of allergies in the triggering of which the major allergen Phl p 1 from timothy grass is involved, by immunotherapeutic DNA vaccination.

30

35